의학석사 학위논문

Pharmacokinetic Analysis of Etanercept Following a Single Subcutaneous Dose in Healthy Volunteers: a Combined Analysis of Five Clinical Trials

건강한 한국인 자원자에서 **Etanercept** 단회 피하투여에 의한 약동학적 특성에 관한 연구

2014년 2월

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의학과 약리학 전공

안 리 영

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이 논문을 의학석사 학위논문으로 제출함

2013년 10월

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안리영의 의학석사 학위논문을 인준함

2013년 12월

위 원 장 예 상 규 (인)

부위원장 장 인 진 (인)

위 원 유 경 상 (인)

Abstract

Pharmacokinetic Analysis of Etanercept Following a Single Subcutaneous Dose in Healthy Volunteers: a Combined Analysis of Five Clinical Trials

Li Young Ahn

Department of Pharmacology, College of Medicine

The Graduate School

Seoul National University

Introduction: Etanercept is a soluble recombinant human tumor necrosis factor receptor fusion protein which is used for the treatment of rheumatoid arthritis and other inflammatory diseases. The aim of this study was to identify the factors that affect the pharmacokinetics (PK) of etanercept by

comparing the results of PK analysis from five clinical trials.

Methods: The serum etanercept concentration data of 169 healthy subjects from five clinical trials were pooled for both noncompartmental and compartmental analyses. Serial blood samples were collected up to 3 or 4 weeks after a single subcutaneous administration of etanercept 25 mg. Enzyme-linked immunosorbent assay (ELISA) was used to determine the serum concentrations of etanercept.

Results: Noncompartmental analysis showed significant differences in PK parameters such as the maximum concentration, area under the time-concentration curve, and half-life, among the five trials. Population PK analysis demonstrated that PK parameters were influenced by formulation, body weight and the study effect. Differences in the distribution of PK parameters among the five clinical studies were attributed to differences in bioanalytic methods, manufacturing batch variability of etanercept, and changes in the manufacturing process.

Conclusion: Considering the complexity of protein products, identifying variations is important and these results may contribute to deepening our understanding of such variability.

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Key words: etanercept, biologics, pharmacokinetics, TNF inhibitor

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INTRODUCTION

Tumor necrosis factor (TNF) is a cytokine that is involved in the development and maintenance of the immune system [1]. It has been implicated in the pathology of a variety of diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), and psoriasis [2-4]. Binding of an antibody to TNF can inhibit or prevent the interactions of this cytokine with its cellular receptors and may prevent the effects caused by excessive TNF.

Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding domain of the 75kD receptor for TNF fused to the Fc portion of the human immunoglobulin G1 [5]. The drug inhibits the activity of TNF by competitively binding to this proinflammatory cytokine and preventing interactions with its cell surface receptors [6, 7]. Etanercept was approved as Enbrel® (Pfizer, New York, USA) by the Food and Drug Administration of the United States in 1998. Etanercept is indicated for the treatments for RA, juvenile rheumatoid arthritis (JRA), psoriatic arthritis, AS, and psoriasis [5, 8, 9].

The pharmacokinetics (PK) of etanercept has been well studied using both compartmental and noncompartmental methods in diverse populations of healthy subjects and patients [10-14]. Etanercept is probably the TNF inhibitor with the best characterized PK properties than other currently marked TNF inhibitors (eg., influximab, adalimumab). Etanercept is slowly

absorbed after single subcutaneous (SC) dose with a peak concentration time of 50 to 60 hours and eliminated with a mean half-life ($t_{1/2}$) approximately 68 hours in healthy volunteers [10]. After a single 25 mg subcutaneous injection of etanercept to RA patients, the $t_{1/2}$ was 102 ± 30 hours [15]. When the data from children with JRA were analyzed, gender and body surface area were significant covariates on clearance, whereas the volume of distribution was affected by body weight in a one compartment model [13]. When a population PK analysis was conducted on the combined data in healthy subjects, patients with RA and AS, a two compartment structure model was identified and age, body weight, and race were found to be important covariates [11, 16].

However, anti-TNF agents, including etanercept, are not effective in all patients. About 30% of patients treated with a TNF inhibitor failed to achieve an improvement of 20% in American College of Rheumatology criteria (ACR 20), and more patients lose efficacy during therapy or experience adverse events following treatment with a TNF inhibitor [17]. Moreover, serum concentrations of TNF inhibitors have been shown to be highly variable between individuals and differ over time even within an individual patient [18]. The inter-individual variability of the concentration-time profiles and the exposure characteristics can be explained by the patient and disease characteristics, including concomitant use of immunosuppressives, serum albumin concentration, and the degree of systemic inflammation. Patients with a baseline serum albumin concentration below normal range, a common finding associated with severe inflammation, have lower remission rates after treatment with infliximab that suggested an inverse relationship

between serum albumin concentration and infliximab clearance in CD and ulcerative colitis patients [19, 20]. Gender has also been shown to independently influence the disposition of infliximab with clearance being higher in men [18].

Another factor that affects the PK is the complexity of biologics. In contrast to small molecular drugs, biologics are more complex, difficult to manufacture and have greater process-related variability that can impact efficacy and safety [21]. It is difficult to avoid heterogeneity between batches from the same manufacturing process and small changes in, or differences between, manufacturing processes may have a significant impact on the quality, purity, biological characteristics and clinical activity of the final product [22].

Collectively, these factors probably account for the large inter-individual differences in PK and clinical efficacy observed after standard dosing of TNF inhibitors. However, most PK studies report a large inter-individual variability in which sources are not identified [23]. In a study of infliximab, marked differences in exposure between RA patients with trough serum concentrations varying more than 100-fold in the group receiving 3 mg/kg every 8 weeks and reported that it was not able to explain such a variability [24].

PK variability has clinical consequences because relationships between biologics concentrations and effects are reported. An association between low circulating drug levels and lack of clinical response was observed for infliximab and adalimumab treated patients [25, 26]. Also,

etanercept showed a significant association between clinical response and serum etanercept levels in RA patients [27]. Therefore, understanding factors that influence the concentration-time profiles and the exposure characteristics is essential to further improve the therapeutic efficacy of this drug.

In this study, we evaluated the PK of etanercept of five clinical trials of etanercept in healthy Korean subjects by both noncompartmental and compartmental methods and investigated the factors that influence the PK of etanercept by comparing the PK parameters of each clinical study.

MATERIALS AND METHODS

Source of pharmacokinetic data

The serum concentration data of etanercept for healthy subjects were pooled from five different clinical studies which were conducted during 2008 to 2013. All studies were a bioequivalence study which were a double-blind, randomized, two-sequence, two-period, two-treatment, crossover, single-dose study and compared the test drug with the reference drug (Enbrel®) of etancercept 25 mg in healthy male subjects. The datasets were prepared from the serum etanercept concentration data of the reference drug of each study. The protocols of all studies were approved by the Institutional Review Board at Seoul National University Hospital (SNUH) and conducted at the Clinical Trials Center of SNUH.

However, as shown in Table 1, differences of formulation of the drug, number of subjects, PK sampling times, study designs (wash-out period), and the institute of the serum concentration measurement existed among five studies. Studies 1 and 2 used etanercept in vials containing 25 mg lyophilized powder requiring reconstitution, and the other studies, studies 3, 4, and 5 used the 25 mg/mL liquid formulation supplied in a prefilled syringe. Etanercept was subcutaneous (SC) administered at the right or left upper quadrant of the abdomen in all studies.

The serum concentrations of etanercept were determined using

validated enzyme-linked immunosorbent assay (ELISA) procedures in all studies. However, the ELISA commercial diagnostic kit, bioanalyic institutions, and the lower limit of quantification for etanercept were different among studies.

Subjects

The demographic inclusion criteria for healthy Korean male subjects are summarized in Table 2. Subjects provided written informed consent before being screened for all studies and were determined to be healthy based on medical history, physical examination, vital signs, 12-lead electrocardiography (ECG), serology (HBsAg, anti-HCV and anti-HIV antibody), routine clinical laboratory tests (clinical chemistry, hematology and urinalysis) and urinary drug screening (amphetamine, cocaine, barbiturates, benzodiazepine and opioids) performed within the three weeks preceding the start of this study. Subjects were also excluded if they had used any prescription medication or herbal medicines within the two weeks preceding the study or if they had taken any over-the-counter medication or vitamin beverages within a week of the study.

Noncompartment pharmacokinetic assessment

The individual PK properties of etanercept were analyzed using a

noncompartmental method with WinNonlin® (Version 6.3, Pharsight Corporation, CA, USA). Actual blood sampling times were used in the analysis. The maximum drug concentration in plasma (C_{max}) and the time to reach C_{max} (t_{max}) were obtained directly from the observed values. The terminal elimination rate constant (λ_z) was estimated using linear regression of the log-linear decline of individual plasma concentration-time data. The individual half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$, where ln is the natural logarithm. The area under the concentration-time curve (AUC) from time 0 to the last observation (AUC_{0-t}), was calculated using the linear-up and log-down trapezoidal method in plasma concentration-time curves. Since the sampling times were different among studies, the AUC from time 0 to 480 hours (AUC_{0-480h}) was calculated to compare the exposure of each study. The AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated by adding C_{last}/λ_z to AUC_{0-t}, where C_{last} is the last measurable concentration. Oral clearance (CL/F) was calculated as Dose/$AUC_{0-\infty}$.

Population pharmacokinetic model development

A population PK analysis was conducted using NONMEM (Version 7.2, Icon Development Solutions, Ellicott City, MD, USA) with the G77 FORTRAN compiler. The first-order conditional estimation method with η−ε interaction was used throughout the model development. One− or two−compartment models with first-order or zero-order absorption were evaluated to identify the

one which best described the serum concentration−time profiles of etanercept. Inter−individual variability (IIV) of PK parameters was evaluated using exponential error model, and the PK parameters of the i^{th} subject (P_i) were described as the following equation:

$$P_i = \theta \cdot \exp(\eta_i) \quad (1)$$

Where θ is the typical value of the PK parameters, and η_i is a random variable of the i^{th} subject. The correlation between the IIV in apparent clearance (CL/F) and apparent volume of distribution (V/F) was examined using the $OMEGA BLOCK option. An additive, a proportional, or a combined additive and proportional error model was tested for residual error.

Model selection was based on the precision of parameter estimates, goodness-of-fit plots, as well as on significant decrease of the objective function value (OFV) provided by NONMEM. A decrease of 3.84 in OFV (α = 0.05) for two nested models differing by one parameter was considered significant.

Categorical variables such as formulation (FORM), analytical method, study (STU), period and continuous variables such as age, height, body weight (WT), ideal body weight, body mass index were tested as potential covariates. The covariate screening procedures were performed using visual (scatter plots for continuous variables and boxplots for categorical variables) and numerical (Akaike information criteria in generalized additive model) approaches. Covariates that passed the screening procedures were explored in a stepwise fashion with forward selection (α =

0.05, df = 1) and backward elimination (α = 0.01, df = 1).

Model evaluation

The stability and robustness of the final population PK parameter for etanercept model were evaluated as internal validation by using a bootstrap method. 1,000 bootstrap datasets were generated from the original dataset and the parameter estimates for each dataset were estimated using the final population PK model. The median and 95% confidence intervals (CIs) (2.5th and 97.5th percentiles) of the bootstrapped parameter estimates were obtained and compared with the final PK parameter estimates. In addition, visual predictive checks (VPCs) were performed by using 1,000 datasets simulated from the final PK model. The median and 90% CIs (5th and 95th percentiles) of etanercept serum concentrations at each observation time in the simulated dataset were overlaid with observed data classified by STU and WT.

Statistical analysis

All statistical analyses were performed using SAS® version 9.3 (SAS Korea). Demographic characteristics and PK parameters were presented as descriptive statistics and demographics and PK parameters (C_{max}, AUC_{0-480h}, $AUC_{0-\infty}$, and $t_{1/2}$) were compared between studies using analysis of variance (ANOVA). C_{max} and AUC_{0-480h} were compared within same formulations by t-test or

ANOVA. A P-value less than 0.05 were considered to be statistically significant.

RESULTS

Subjects

The development dataset contained data from 169 healthy male subjects, which yielded a total of 2,637 serum etanercept observations. Subjects from five studies aged 20 to 42 years (mean [SD], 26.5 [4.6] years) and weighing 50.2 to 93.7 kg (68.3 [8.0] kg). The demographic data of each study are summarized in Table 1 and age, height, and weight were not significantly different between studies (ANOVA, P-value: age, 0.196; height, 0.899; weight, 0.814). There were no notable differences in baseline characteristics among study groups.

Table 1 Study design and demographic data

	Study 1	Study 2	Study 3	Study 4	Study 5
Formulation	Reconstituted	Reconstituted	Liquid	Liquid	Liquid
Wash-out (days)	28	28	28	28	35
PK sampling time (h)	0, 3, 6, 12, 24, 36, 48, 60, 72, 96, 144, 216, 312, 480h	0, 3, 6, 12, 24, 36, 48, 60, 72, 96, 144, 216, 312, 480h	0, 3, 6, 12, 24, 36, 48, 60, 72, 96, 144, 216, 312, 480h	0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 216, 288, 384, 504h	0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 216, 288, 384, 504, 648h
Bioanalytic institute (LLOQ)	I (50 ng/mL)	II (97.7 ng/mL)	II (97.7 ng/mL)	III (100 ng/mL)	III (80 ng/mL)
Duration of the Study	Nov, 2008 - Feb, 2009	Jan, 2010 - Jul, 2010	Sep, 2011 - Mar 2012	Mar, 2010 - Jan, 2011	Nov, 2012 - Mar 2013
Number of subjects (n)	19	35	39	33	43
Age (years)	26.2 ± 4.7 [22 - 42]	25.0 ± 3.9 [21 - 36]	26.7 ± 4.2 [21 - 37]	26.6 ± 3.8 [20 - 38]	27.6 ± 5.8 [20 - 41]
Height (cm)	174.2 ± 4.6 [165 - 182]	173.7 ± 5.9 [160.4 - 184.2]	174.7 ± 4.4 [165.6 - 184.3]	173.7 ± 5.5 [164.9 - 186.1]	173.9 ± 5.8 [163.6 - 190.8]
Weight (kg)	69.9 ± 9.4 [54.6 - 88.8]	67.8 ± 6.1 [57.0 - 79.2]	69.0 ± 8.1 [55.8 - 84.7]	67.5 ± 8.1 [50.2 - 93.7]	68.1 ± 8.7 [52.8 - 91.7]

Age, height, weight are presented as mean ± SD [range]. Bioanalytic institute are presented as I, II, and III.
LLOQ, lower limit of quantification

Pharmacokinetics of etanercept

Mean serum etanercept concentration versus time curves for subjects in each study are presented in Figure 1. Following SC administration, etanercept showed slow absorption and elimination PK profiles in all studies with a mean $t_{1/2}$ of 122 hours. Especially, a rapid elimination phase was observed in study 1 than other four studies. The mean $t_{1/2}$ of studies 2 - 5 and study 1 was 126.5 hours and 86.6 hours, respectively.

Table 2 summarizes the PK parameters that were derived using the noncompartmental PK analysis approach. The inter-individual variability was 55% for C_{max} and 45% for $AUC_{0\text{-}480h}$ in the total dataset and both mean C_{max} and $AUC_{0\text{-}480h}$ showed an approximately 1.5 fold difference between study 1 and study 4. The PK parameters, C_{max}, $AUC_{0\text{-}480h}$, $AUC_{0\text{-}\infty}$, and $t_{1/2}$, showed a significant difference among five studies (ANOVA, $P<0.05$).

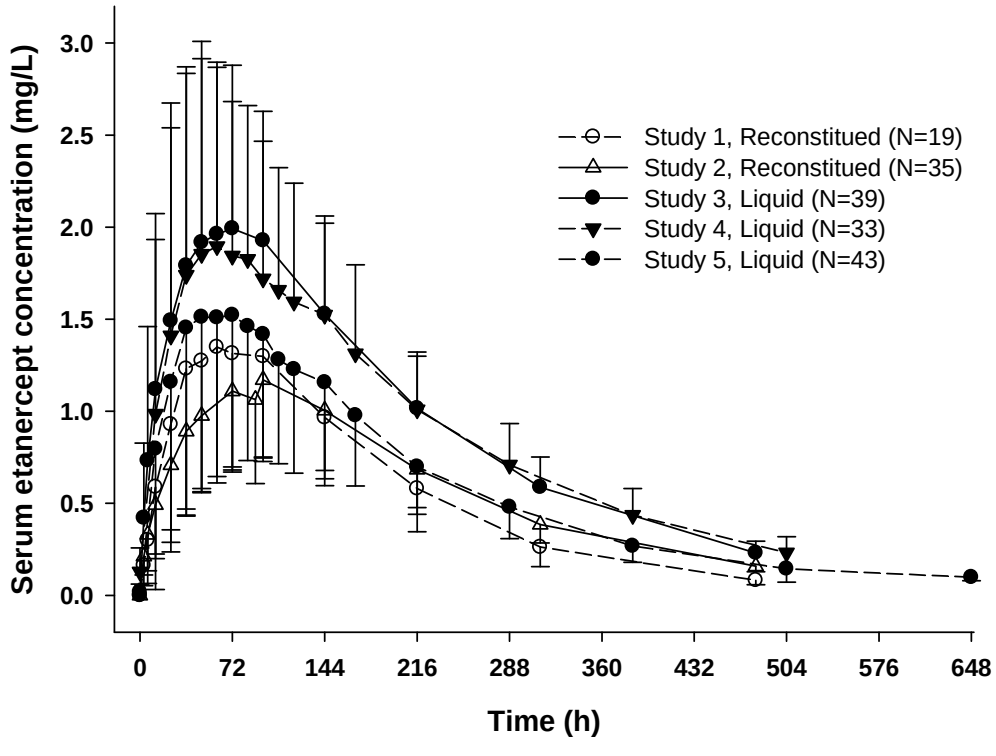
When compared by formulations of etanercept, a greater exposure of liquid prefilled formulation (studies 3, 4, and 5) was observed than reconstituted formulation (studies 1 and 2). The C_{max} and $AUC_{0\text{-}480h}$ were 1.3 ± 0.6 mg/L and 275.6 ± 102.5 h*mg/L in reconstitution formulation and 2.0 ± 1.1 mg/L and 418.7 ± 172.8 h*mg/L in liquid prefilled formulation, respectively (Figure 3).

The C_{max} and $AUC_{0\text{-}480h}$ of study 1 and 2, which administered reconstituted formulation, were similar as the P-value analyzed by t-test was 0.256 and 0.801, respectively. When the PK parameters of studies 3, 4, and 5,

which administered liquid prefilled formulation, were compared by ANOVA and the difference in C_{max} was not significantly different (P=0.080), while $AUC_{0\text{-}480h}$ was significantly different (P<0.001).

The body weight and $AUC_{0\text{-}480h}$ of etanercept showed a negative relationship, however the negative trend decreased when the relationship between ideal body weight and $AUC_{0\text{-}480h}$ was evaluated as shown in Figure 4.

(a)



(b)

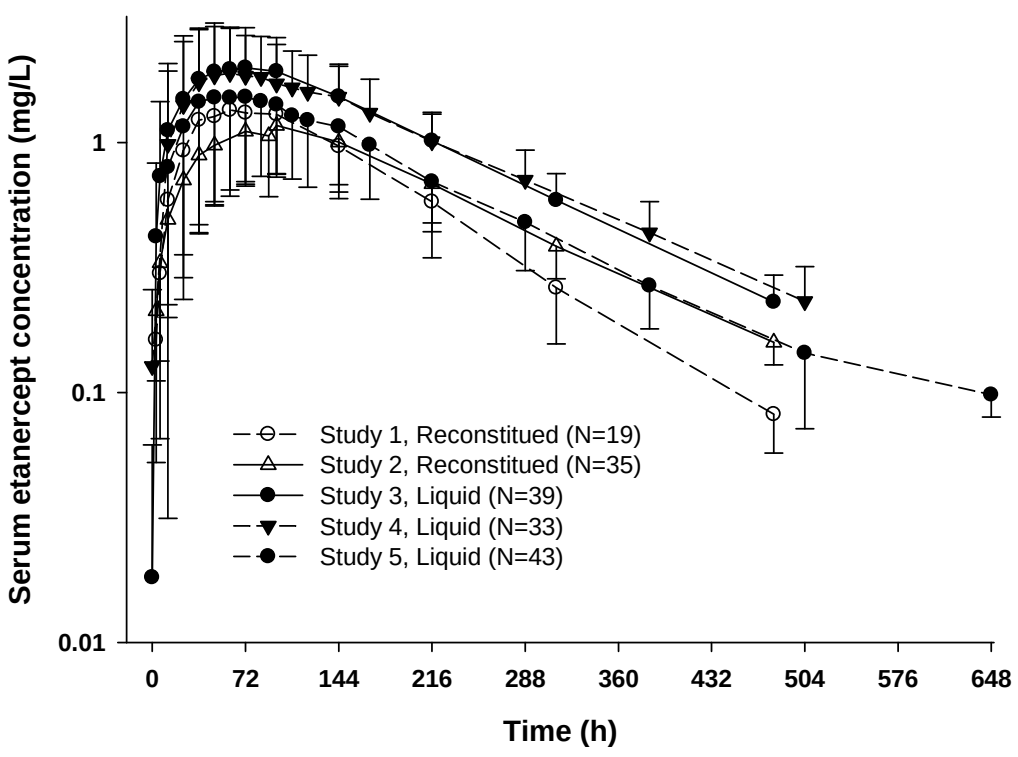
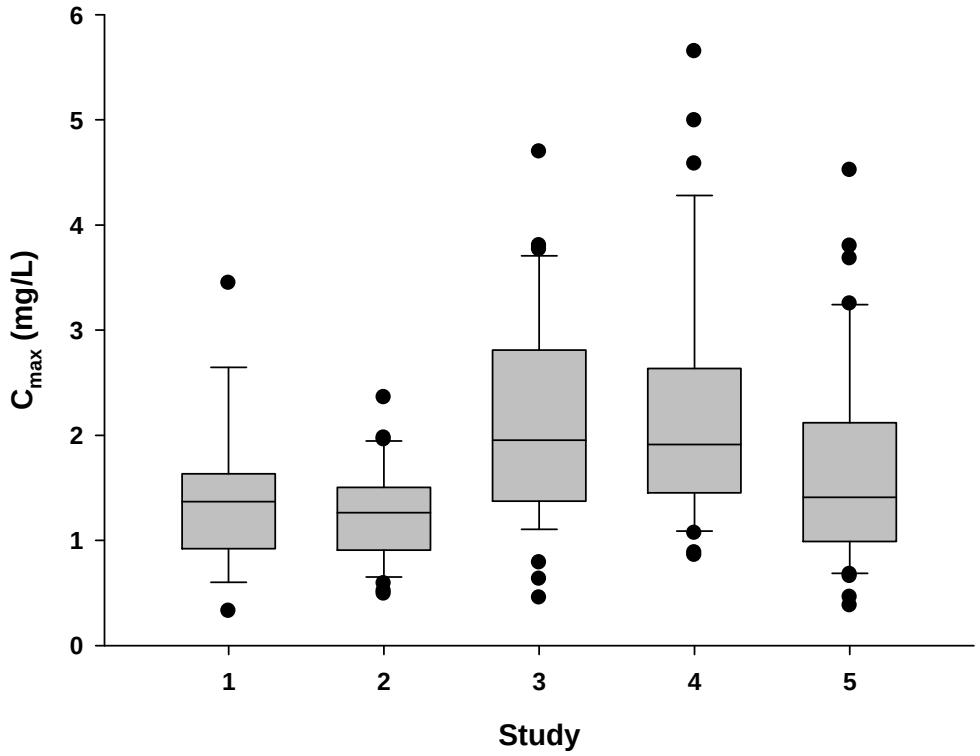


Figure 1 Mean serum concentration versus time profiles of etanercept. (a: linear, b: log-linear). Error bars indicate the standard deviation.

(a)



(b)

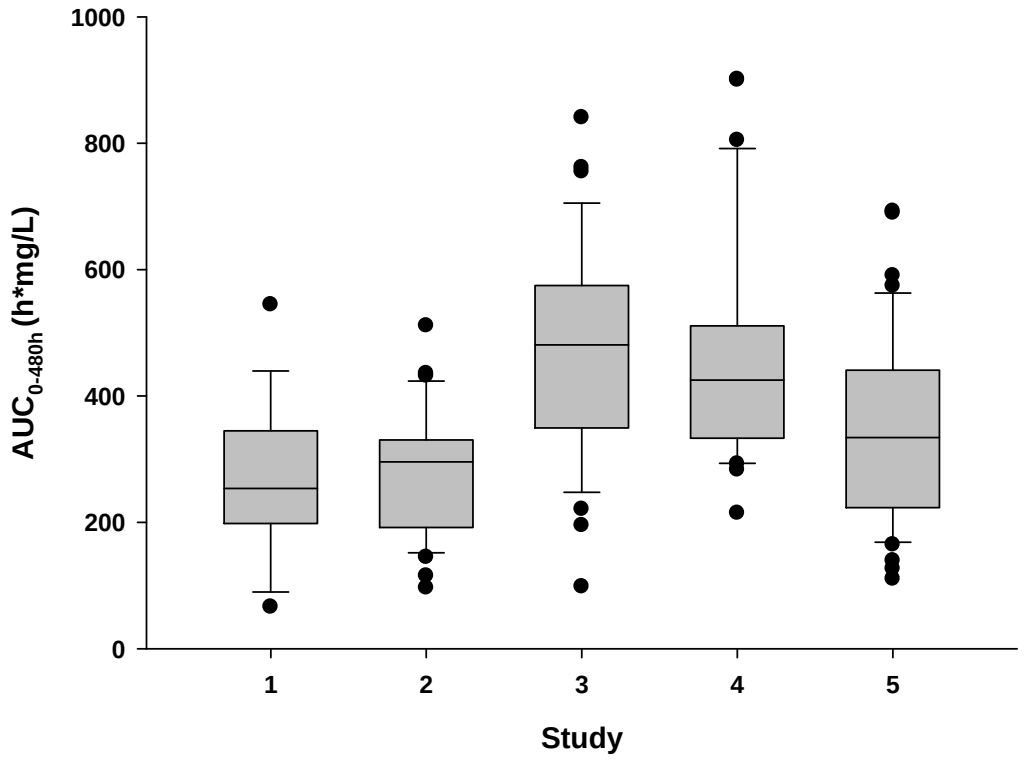
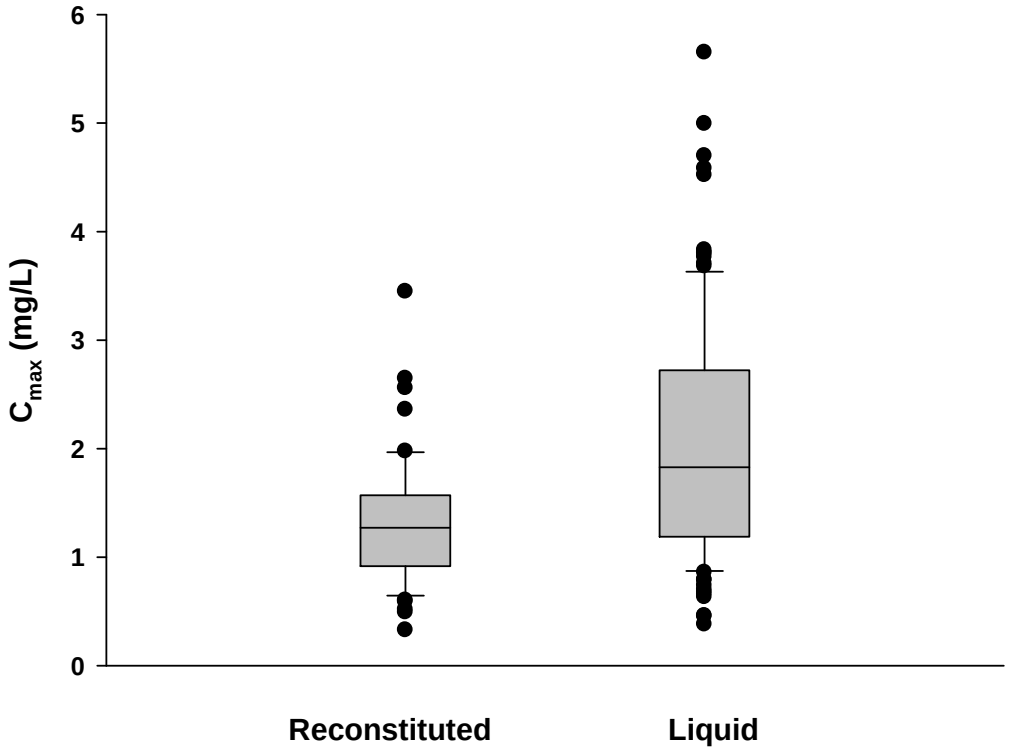


Figure 2 (a) C_{max} and (b) $AUC_{0\text{-}480h}$ of each study after single subcutaneous administration of etanercept 25 mg .The box represents the median and 25th (lower line)-75th (upper line) percentiles. C_{max} maximum concentration of etanercept, $AUC_{0\text{-}480h}$ area under the concentration-time curve from time zero to 480 hours after dose

(a)



(b)

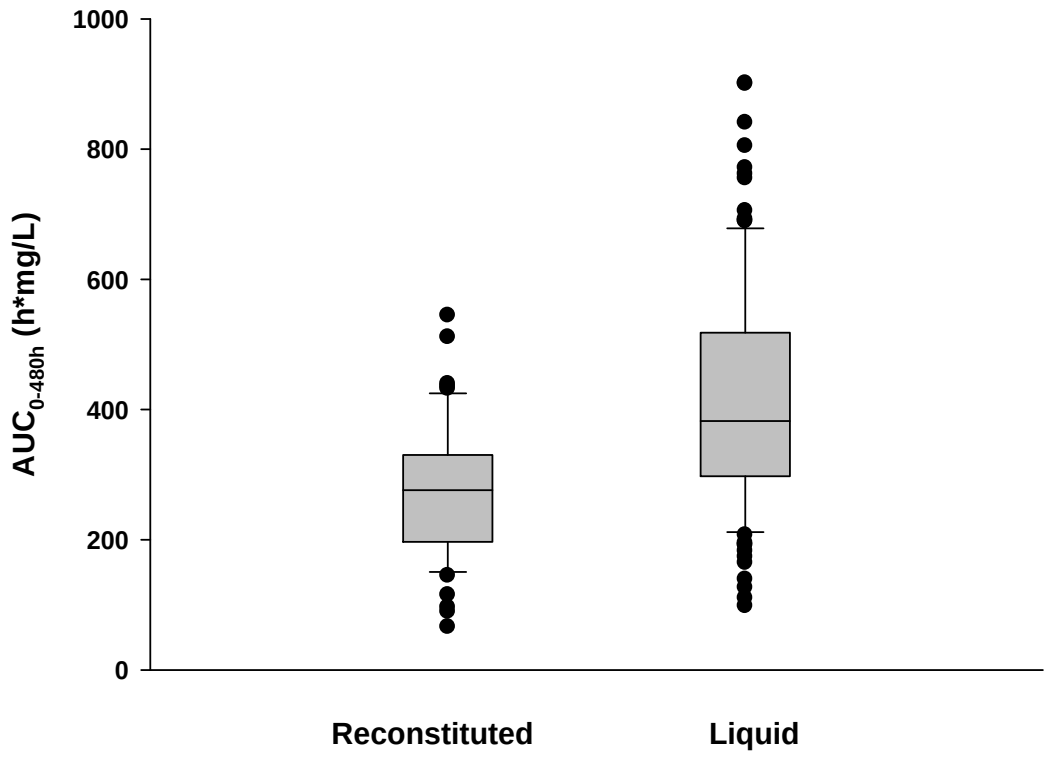
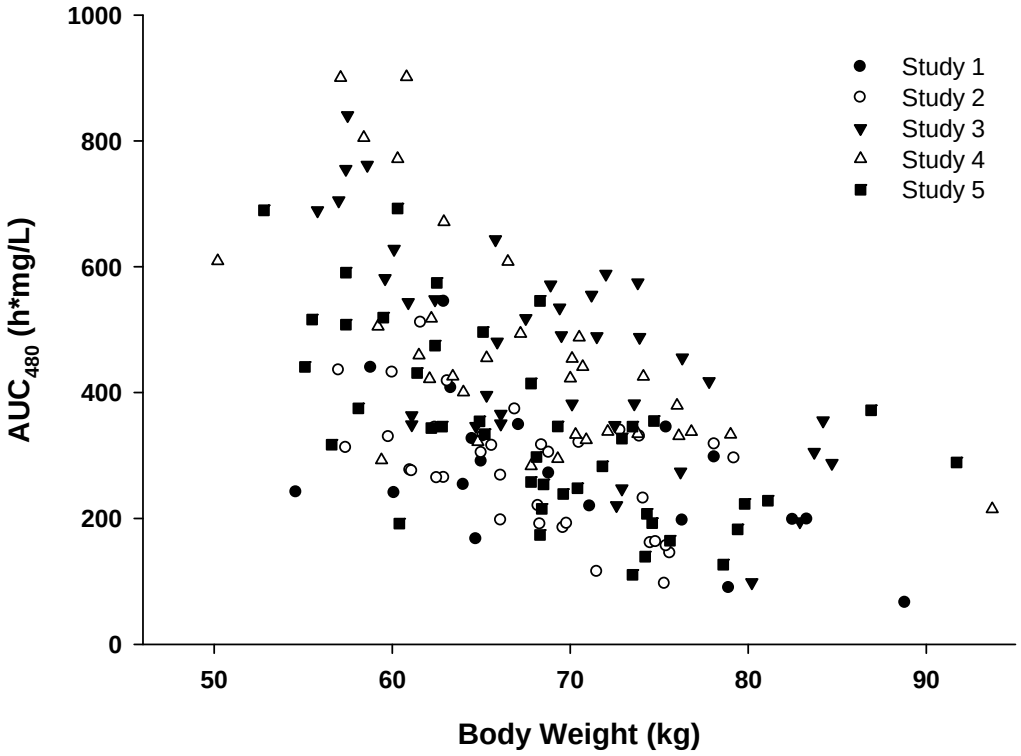


Figure 3 (a) C_{max} and (b) $AUC_{0\text{-}480h}$ after single subcutaneous administration of etanercept 25 mg compared by formulation of etanercept. The box represents the median and 25th (lower line)-75th (upper line) percentiles. C_{max} maximum concentration of etanercept, $AUC_{0\text{-}480h}$ area under the concentration-time curve from time zero to 480 hours after dose

(a)



(b)

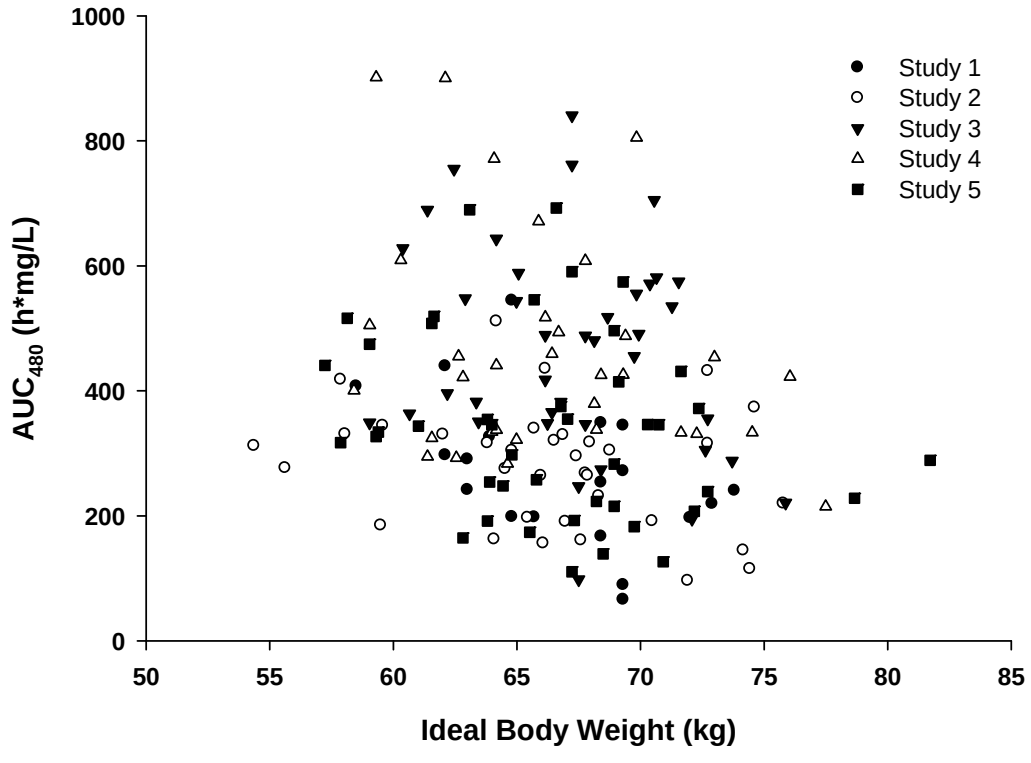


Figure 4 Relationships between (a) $AUC_{0\text{-}480h}$ and body weight, and (a) $AUC_{0\text{-}480h}$ and ideal body weight. $AUC_{0\text{-}480h}$ area under the concentration-time curve from time zero to 480 hours after dose

Table 2 Summary of the pharmacokinetic parameters

	Study 1 (n=19)	Study 2 (n=35)	Study 3 (n=39)	Study 4 (n=33)	Study 5 (n=43)	Total (n=169)
C_{max} (mg/L)	1.47 ± 0.75 (51.28)	1.25 ± 0.45 (36.14)	2.15 ± 0.97 (45.31)	2.19 ± 1.17 (53.45)	1.71 ± 1.00 (58.14)	1.78 ± 0.98 (54.99)
t_{max} (h)	72.00 [36.00-143.87]	95.65 [48.02 - 143.83]	72.00 [24.00 - 144.72]	72.00 [24.00-168.22]	60.00 [24.00-169.17]	72.00 [24.00-169.17]
$t_{1/2}$ (h)	86.60 ± 14.38 (16.61)	125.95 ± 34.46 (27.36)	122.35 ± 21.33 (17.43)	137.94 ± 52.68 (38.19)	122.03 ± 43.59 (35.72)	122.04 ± 39.55 (32.40)
AUC_{0-480h} (h·mg/L)	270.71 ± 116.40 (43.00)	278.18 ± 95.87 (34.46)	464.94 ± 168.38 (36.21)	463.30 ± 176.30 (38.05)	342.64 ± 149.02 (43.49)	372.99 ± 167.49 (44.91)
$AUC_{0-\infty}$ (h·mg/L)	283.64 ± 115.40 (40.68)	314.30 ± 88.52 (28.16)	508.84 ± 168.62 (33.14)	519.67 ± 184.77 (35.56)	370.41 ± 151.95 (41.02)	410.13 ± 174.54 (42.56)
CL/F (L/h)	0.11 ± 0.07 (59.89)	0.09 ± 0.03 (32.19)	0.06 ± 0.03 (45.29)	0.05 ± 0.02 (32.24)	0.08 ± 0.04 (45.52)	0.07 ± 0.04 (52.06)

Data presented as mean ± SD (CV%). C_{max}, maximum plasma concentration; t_{max}, time to reach maximum plasma concentration; $t_{1/2}$, terminal phase half-life; AUC_{0-480h}, area under the concentration-time curve from time zero to 480 hours after dose; $AUC_{0-\infty}$, area under the curve from time zero to infinite; *CL/F*, apparent clearance

The final pharmacokinetic model

A one-compartment first-order elimination model with combined residual errors for etanercept best described the data. The parameters of the basic model were CL/F, V/F, absorption rate constant (ka), and relative bioavailability (RF). The typical value of RF for STU 1, 2, 5 was estimated as 0.707 by fixing RF to 1 for STU 3, 4 with relatively high exposure of etanercept. Because the RF value is not absolute bioavailability but relative fraction between STU, CL/F and V/F for STU 3, 4 were re-estimated as their estimates divided by RF (CL/F/0.707), V/F/0.707). The inclusion of RF improved the model fit and decreased the OFV by 61.5. IIVs were included for all PK parameters. The covariates included in the final model were WT for CL/F and V/F, FORM for CL/F, and STU for V/F. The equations for the covariate model were expressed as follows:

$$CL/F = (0.0619\times(1-FORM) + 0.0518\times FORM) \times (WT/68)\ ^\wedge\ 1.97 \quad (2)$$

$$V/F = (8.35 \text{ for STU } 1, 3, 5 \text{ and } 10.4 \text{ for STU } 2,4\ ) \times (WT/68)\ ^\wedge\ 2.69 \quad (3)$$

When a subject was administered etanercept of reconstituted formulation (FORM = 0) with a WT of 68 kg, the typical value of CL/F was expected to be 0.0619 liters/h. For etanercept of liquid formulation (FORM = 1), the CL/F was decreased to 0.0518 liters/h. For a patient was included in STU 1, 3, 5, weighting 68 kg, the typical value of V/F was estimated to be 8.35 liters. The

V/F for STU 2, 4 was increased to 10.4 liters. The relationship between CL/F and WT and between V/F and WT were explained by the power model normalized to the median WT of 68 kg. The exponents of WT on CL/F, V/F were respectively 1.97, 2.69. The incorporation of each covariate in the PK model decreased the OFV and IIV for the corresponding parameter. All parameter estimates, their relative standard error are summarized in Table 3.

The basic goodness−of−fit plots for the final PK model are shown in Figure 5 and demonstrated that individual predicted concentrations of etanercept agreed well with observed data without systemic bias.

Model evaluation

The median parameter estimates and 95% CIs obtained from 1,000 re-sampled bootstrap datasets are summarized in Table 3. All of the parameter estimates of the final PK model were very similar to bootstrap median and fell in the 95% CIs of the corresponding parameters, indicating that the final model was fairly robust. VPCs of the final population PK model are shown in Figures 6 and 7 and stratified by STU and WT. The model-predicted CIs and medians corresponded adequately to the observed data.

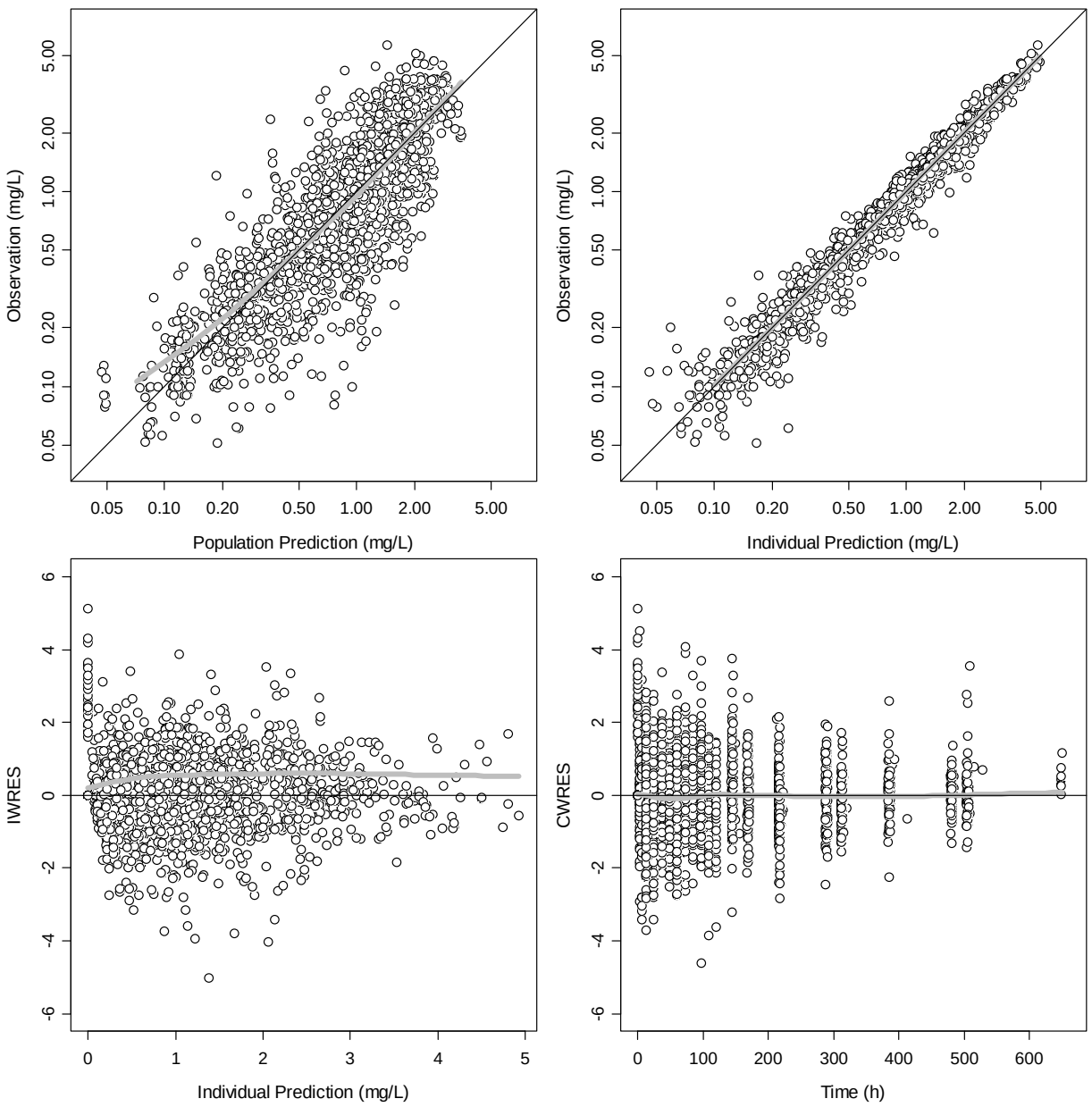
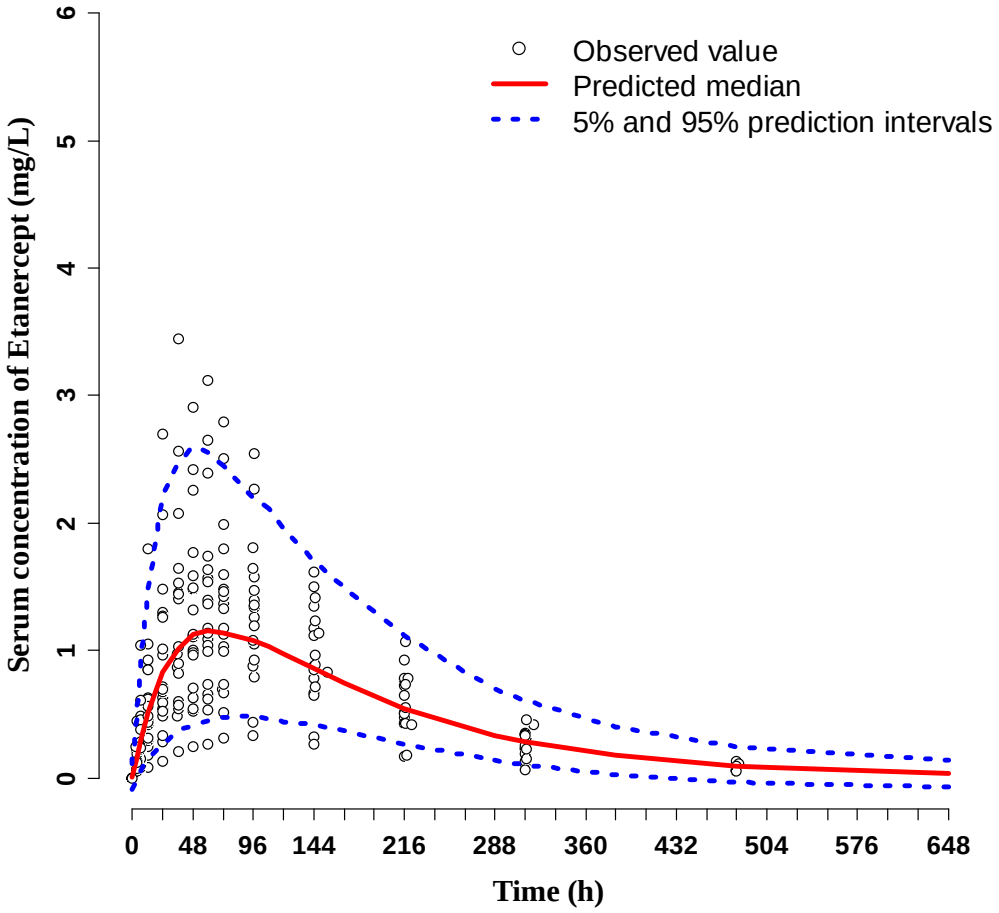
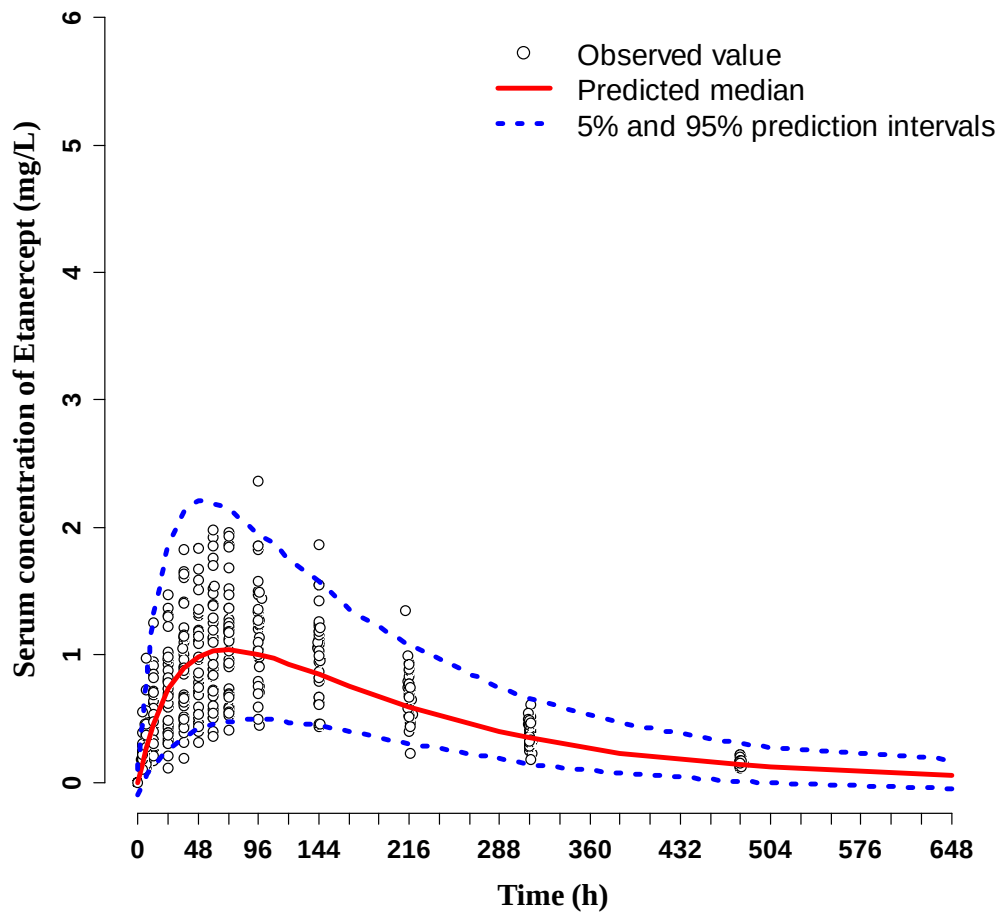


Figure 5 Basic goodness-of-fit plots for the final pharmacokinetic model of etanercept. Black line, line of identity; gray line, locally weighted regression smooth line; IWRES, individual weighted residuals; CWRES, conditional weighted residuals

(a)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
0
1
2
3
4
5
6
0
48
96
144
216
288
360
432
504
576
648
Time (h)

(b)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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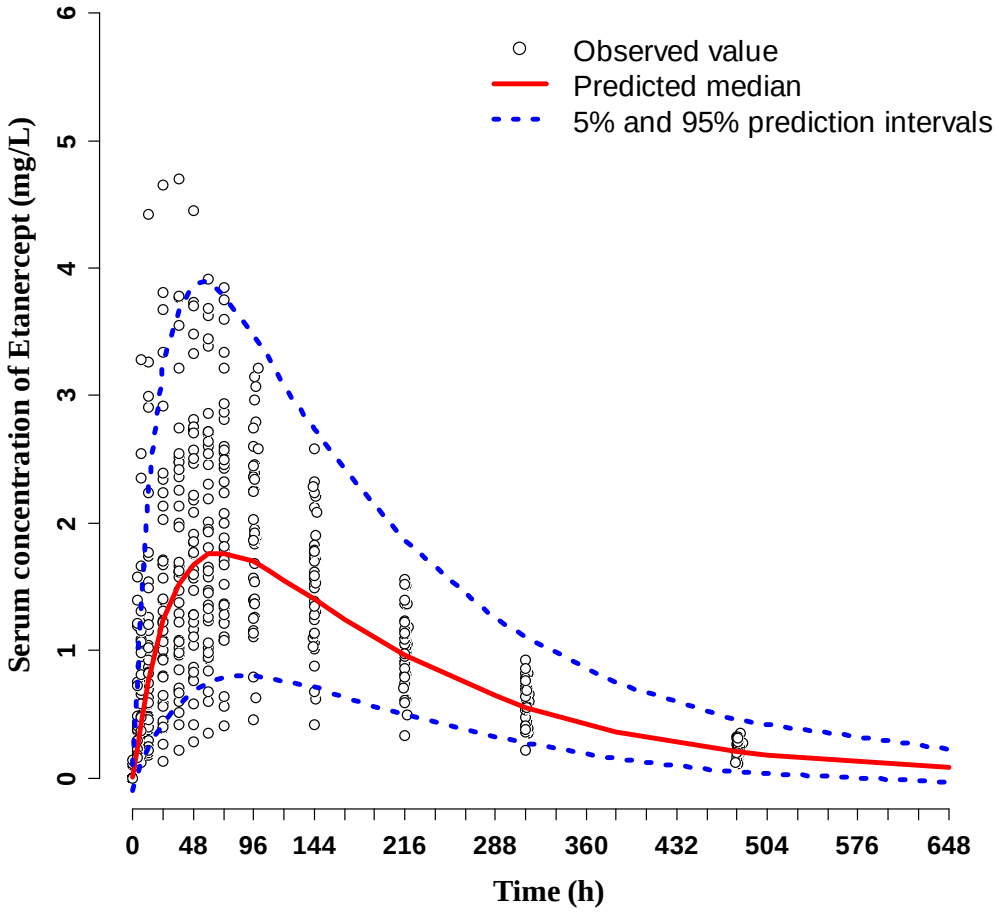
Time (h)

(c)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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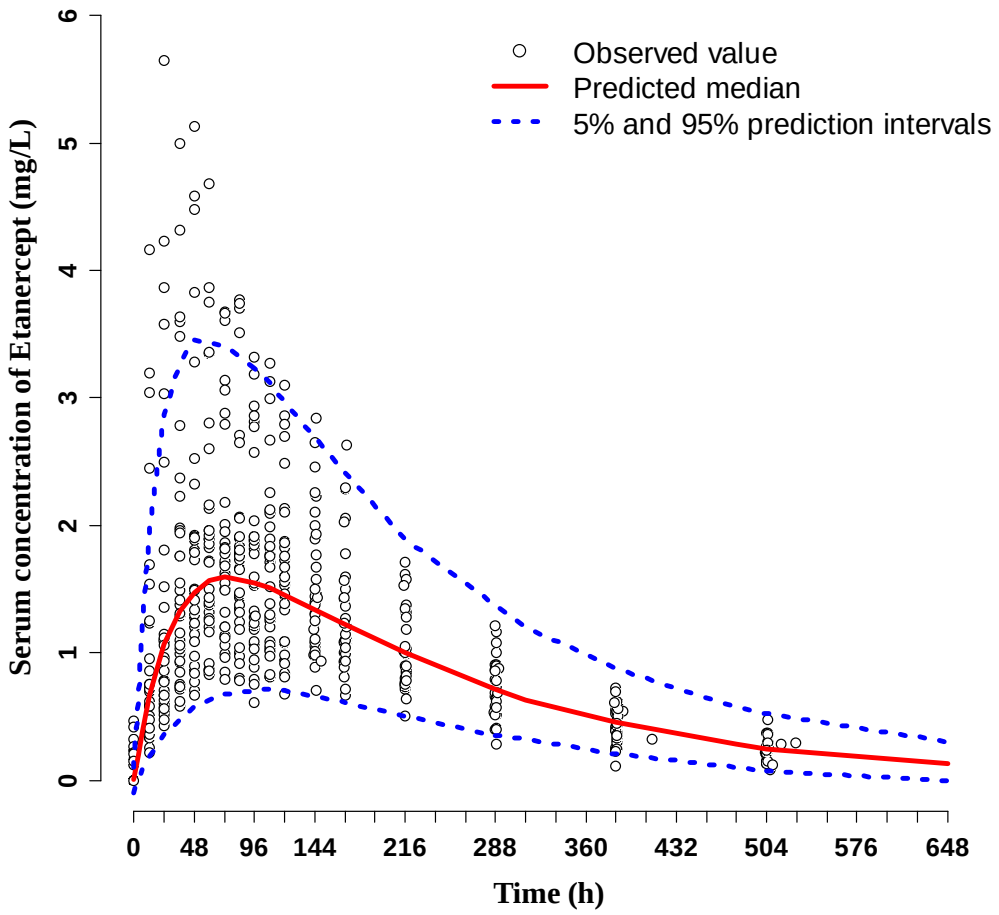
Time (h)

(d)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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Time (h)

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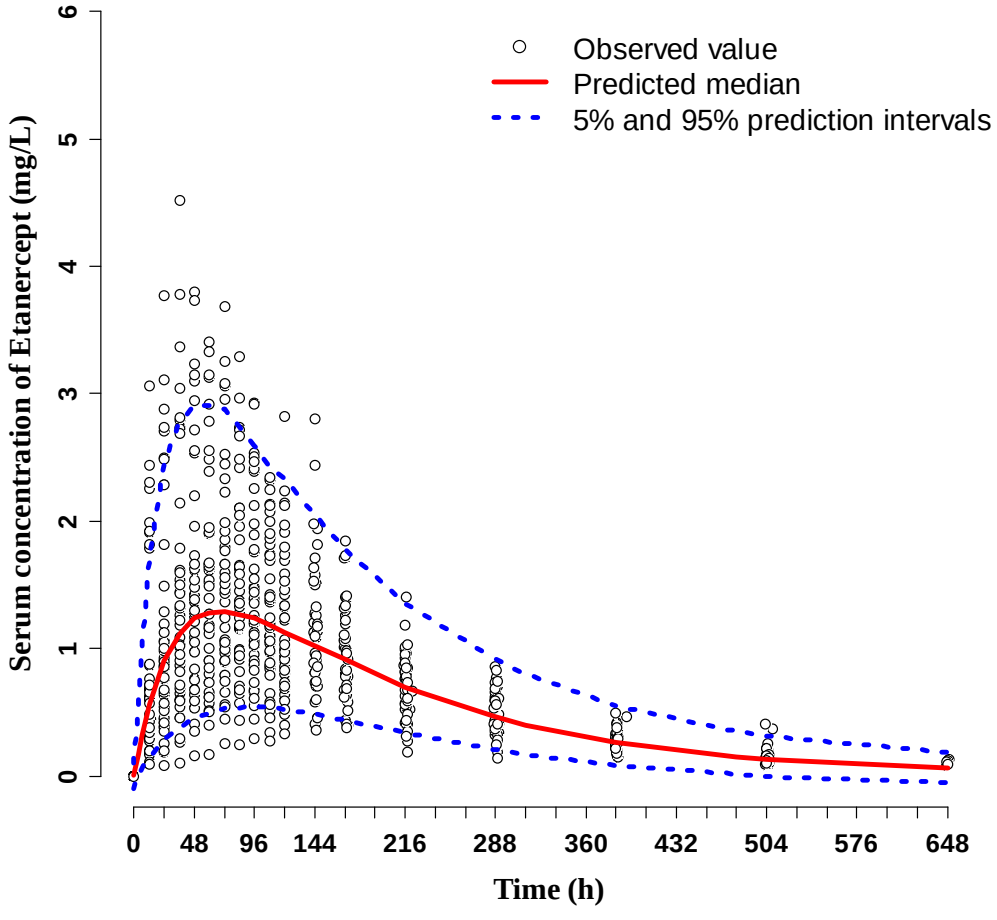
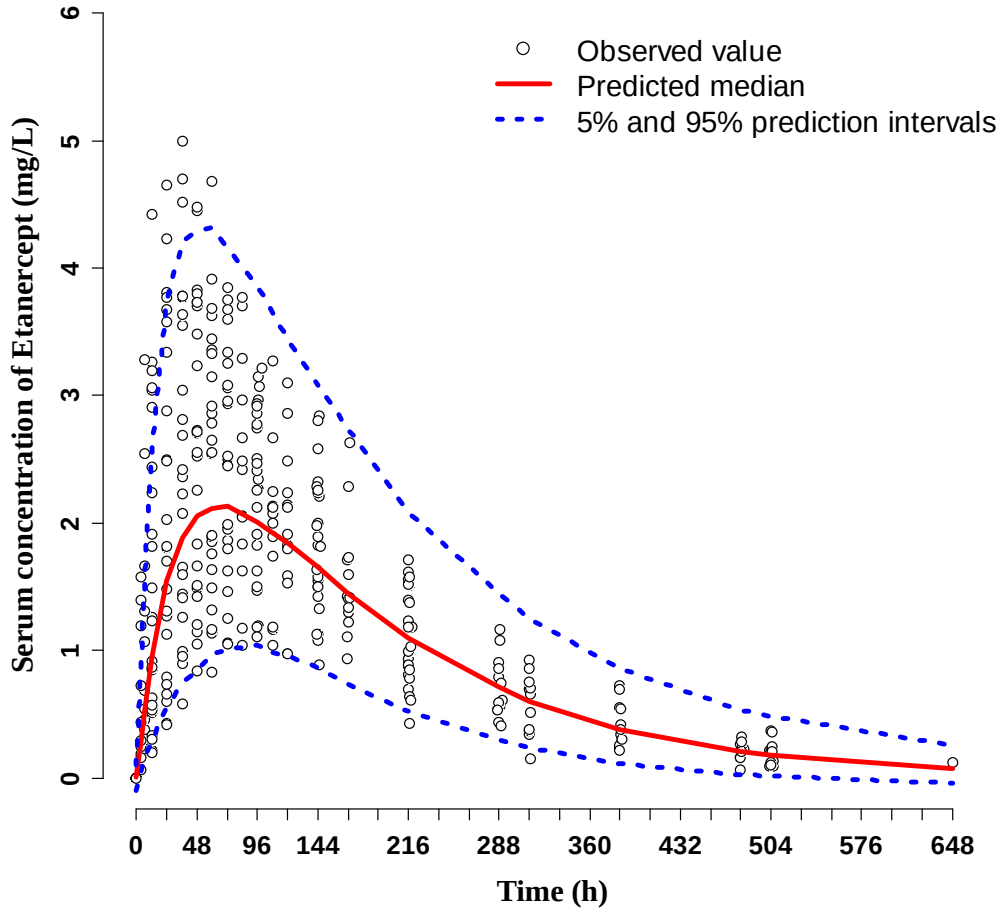


Figure 6 Visual predictive check plots of the final pharmacokinetic model classified by study. (a), study 1; (b), study 2; (c), study 3; (d), study 4; (e), study 5

(a)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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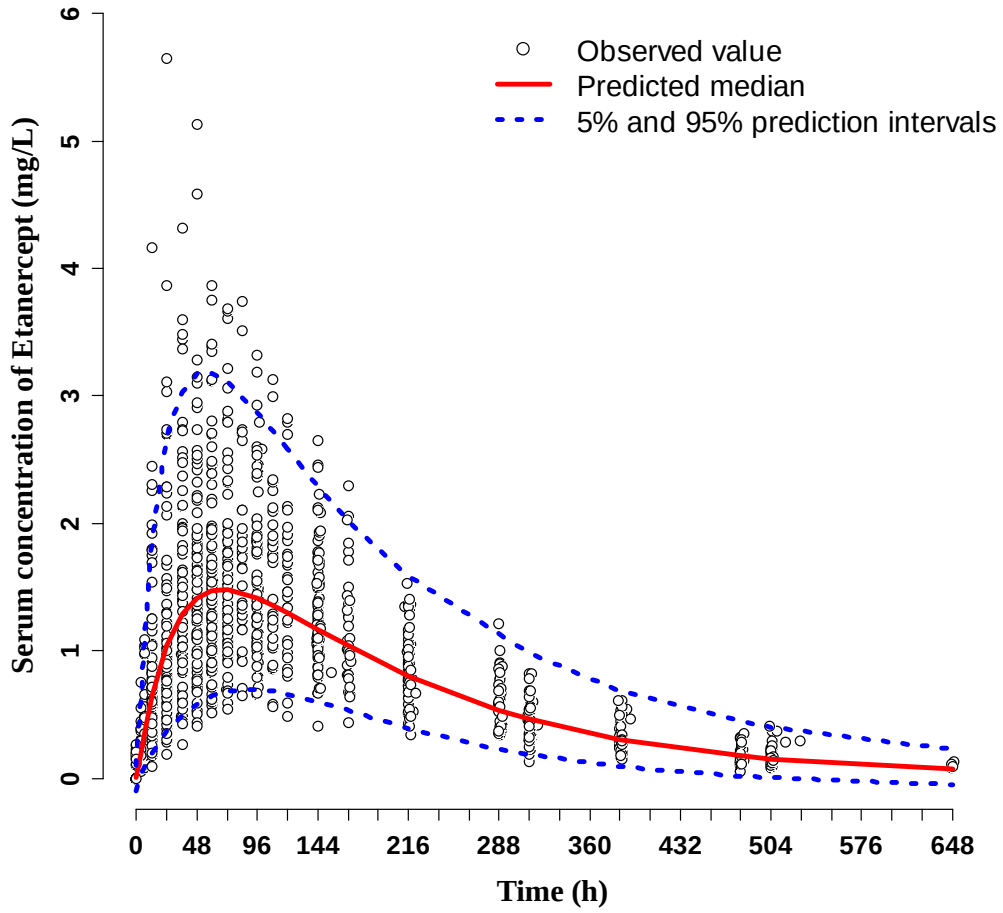
Time (h)

(b)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
0
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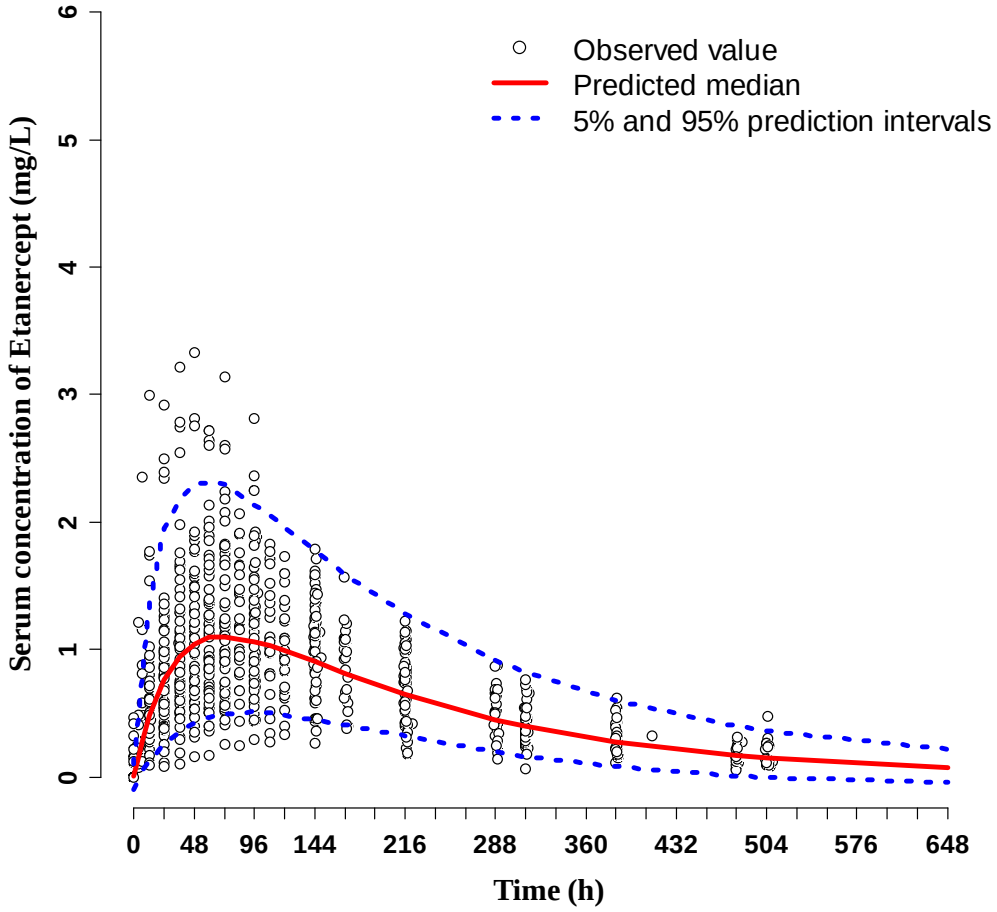
Time (h)

(c)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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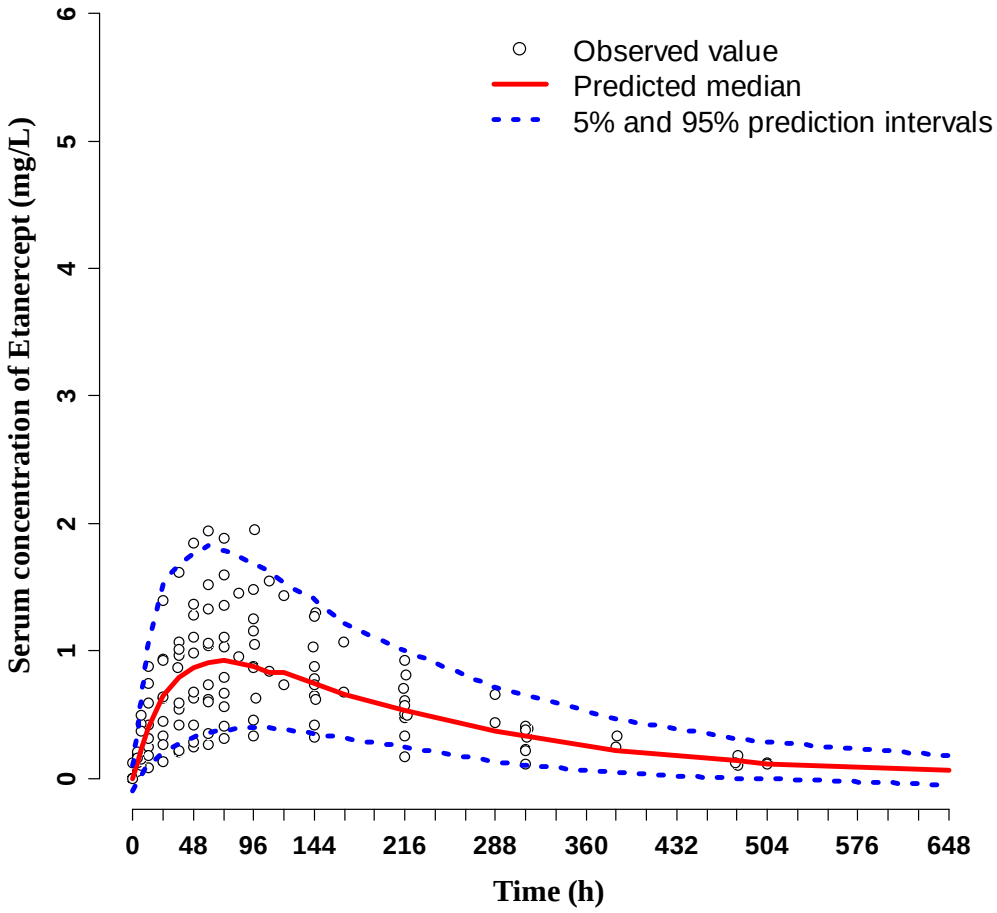
Time (h)

(d)


Observed value
Predicted median
5% and 95% prediction intervals
Serum concentration of Etanercept (mg/L)
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Time (h)

(e)

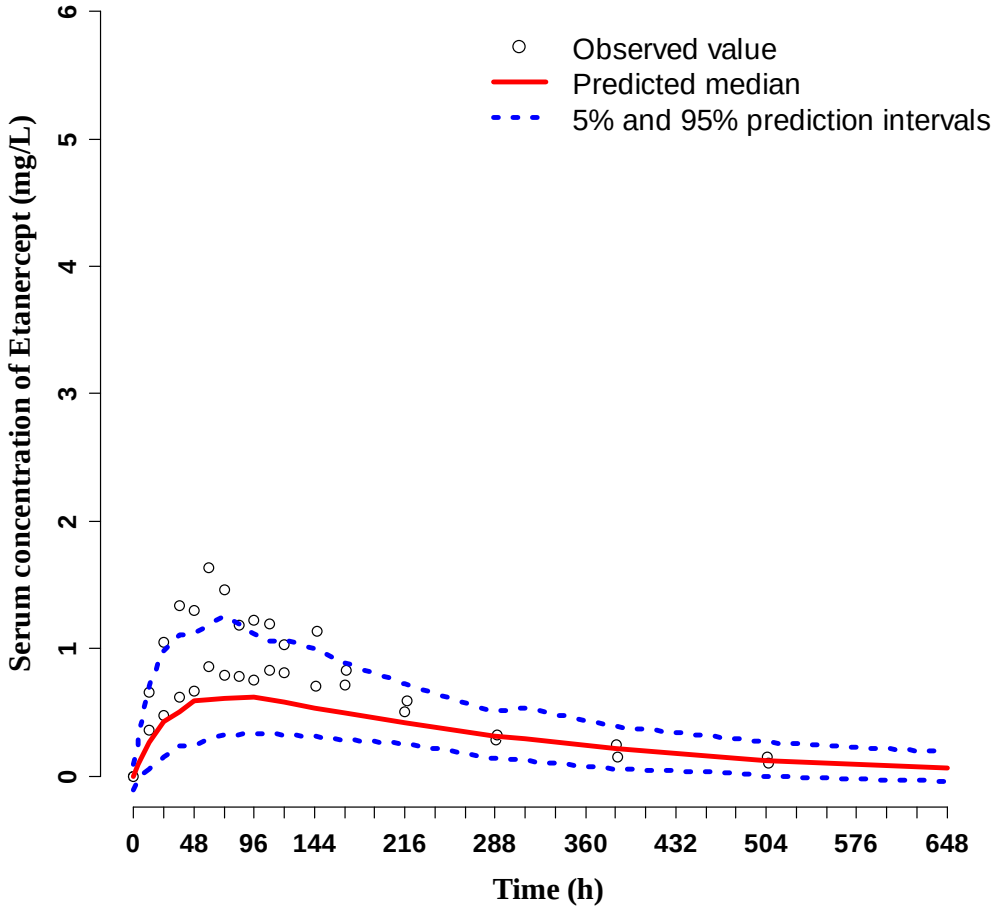

Figure 7 Visual predictive check plots of the final pharmacokinetic model classified by body weight (WT). (a), WT < 60 kg; (b), 60kg ≤ WT < 70 kg; (c), 70kg ≤ WT < 80 kg; (d), 80kg ≤ WT < 90 kg; (e), WT ≥ 90 kg

Table 3 Final parameter estimates and bootstrap results

Parameter	Description (units)	Estimation results Estimate (% RSE)	Bootstrap results Median (95% CI)
Structural model			
$CL/F = (\theta_1 \times (1-FORM) + \theta_2 \times FORM) \times (WT/68)^{\theta_3}$			
θ_1	Apparent clearance for FORM 0 (L/h)	0.0619 (4.81%)	0.0618 (0.0565 − 0.0678)
θ_2	Apparent clearance for FORM 1 (L/h)	0.0518 (3.17%)	0.0518 (0.0488 – 0.0552)
θ_3	Exponent of WT on CL/F (normalized by 68 kg)	1.97 (11.1%)	1.99 (1.56 – 2.41)
$V/F = (\theta_4 \text{ or } \theta_5) \times (WT/68)^{\theta_6}$			
θ_4	Apparent volume of distribution for studies 1, 3, 5 (L)	8.35 (4.01%)	8.34 (7.75 − 9.02)
θ_5	Apparent volume of distribution for studies 2, 4 (L)	10.4 (4.58%)	10.3 (9.54 – 11.4)
θ_6	Exponent of WT on V/F (normalized by 68 kg)	2.69 (11.2%)	2.70 (2.14 – 3.30)
ka	Absorption rate constant (h^{-1})	0.027 (5.15%)	0.027 (0.0244 − 0.0299)
RF	Relative bioavailability for studies 1, 2, 5	0.707 (4.81%)	0.706 (0.638 − 0.780)
Inter-individual variability			
$\omega_{CL/F}$	Inter-individual variability for CL/F (%)	28.5 (11.3%)	28.1 (25.1 – 31.2)
$\omega_{V/F}$	Inter-individual variability for V/F (%)	35.5 (13.7%)	34.9 (30.7 – 39.6)
ω_{ka}	Inter-individual variability for ka (%)	59.7 (12.4%)	59.4 (52.2 – 66.8)
$\rho_{CL/F,V/F}$	Correlation coefficient between CL/F and V/F	0.831 (5.67%)	0.839 (0.723 – 0.916)

Parameter	Description (units)	Estimation results Estimate (% RSE)	Bootstrap results Median (95% CI)
Residual error			
σ_{add}	Additive error (mg/L)	0.0618 (8.93%)	0.0617 (0.0504 − 0.0725)
σ_{prop}	Proportional error (%)	10.2 (6.50%)	10.2 (8.88 − 11.5)

RSE, Relative standard error; FORM, formulation; WT, body weight; RF, relative bioavailability for studies 1, 2, 5 when the bioavailability for studies 3, 4 was assumed to be 1

DISCUSSION

The purpose of this study was to investigate the factors that influence the PK of etanercept by comparing the results of PK analysis from five clinical studies. The PK parameters estimated in the pooled data were within the range of what has been reported previously; after administration of 25 mg of Enbrel® by a single SC injection to 25 patients with RA, a mean ± standard deviation $t_{1/2}$ of 102 ± 30 hours was observed with a C_{max} of 1.1 ± 0.6 μg/mL and time to C_{max} of 69 ± 34 hours [6]. However, PK analysis by both noncompartment and compartment methods demonstrated that PK parameters of etanercept were influenced by the study effect and body weight.

Several reasons for the study effect exist. Differences in number of samples, sampling times, study designs, and experimental conditions for sampling and assaying could be the main sources of inter-study variability (ISV) [28]. In this study, differences of formulations, manufacturing process, and bioanalyic methods were considered to be the main factors that caused ISV of PK parameters among five clinical studies.

Etanercept was originally introduced in vials containing 25 mg lyophilized powder requiring reconstitution and a liquid formulation, supplied in prefilled syringes for SC administration was developed. It is reported that the safety and efficacy of a 50 mg etanercept dose (administered SC once weekly as 2 injections) was comparable to that of 2 separate 25 mg etanercept [14, 29]. In contrast to previous studies, a greater exposure of the liquid

prefilled syringe formulation in comparison with lyophilized reconstituted formulation was observed and the modeling results showed that the difference of CL/F between two formulations was about 20%.

However, limitations exist to conclude that there are clinically significant differences between two formulations. The $AUC_{0\text{-}480h}$ showed a significant difference within the liquid prefilled formulation studies. This was also identified by the difference of RF between two groups in the population PK analysis. The OFV was significantly decreased and the model fit was improved when studies were grouped as studies 1, 2, 5 and studies 3, 4 rather than grouped by formulation. Because, this study was not a controlled study to compare the difference between two formulations and considering the complexity of protein drugs, other factors might had influenced the PK profiles of etanercept. Nowadays, etanercept are on the market as 25 mg, 50 mg single-use prefilled syringe, and 25 mg multiple-use vial [15]. Even though the differences are not clinically significant, clinicians should be aware of the possibility of the difference of drug exposure when considering the interchange of the formulation [30].

From the results of noncompartment analysis, the elimination of the concentration of etanercept of studies 2 - 5 was slower than study 1 and the mean $t_{1/2}$ of study 1 was similar with previous studies investigated in healthy volunteers [10, 31]. This observation can be explained by the change of the manufacturing process. It was reported that the quality profile for batches of Enbrel® having expiry dates after the end of 2009, a changed glycosylation profile appeared on the market in parallel [32]. The expiry date of study drug

used in study 1 was Mar, 2010 and studies 2 - 5 were after Dec, 2010, which suggests the products of studies 2 - 5 were manufactured by the changed quality profile batches. In addition, it has been shown that changes in PK profiles of glycoslyated therapeutic protein occurs through glycosylation heterogeneity and such changes can alter the biological activity of the protein [21, 33].

The manufacture of biological products is a complex process that involves continual refinement throughout product development, post-approval and marketing [21]. In addition, lot-to-lot variability in product quality is observed commonly, even when manufacturing has been performed using the same process [34]. Thus, patients with treatments for a long-term, continuous monitoring are necessary because any change in manufacturing likely to affect the product including the PK parameters. In addition, the difference of the bioanalyic methods among five studies was considered to be another factor that caused ISV by the difference of the commercial ELISA kit.

The pharmacology of therapeutic proteins is complex and depends not only on the product but also on the patient- and disease-related factors. In this study, body weight was a significant covariate for the PK of etanercept in the pooled data analysis. As body weight increased the AUC decreased and this was also identified by the population PK analysis as both CL/F and V/F showed a positive relationship with WT which is consistent with previous population PK models of etanercept [16]. The body weight was also shown to be a significant covariate for adalimumab and infliximab [35, 36].

However, as shown in Figure 4, $AUC_{0\text{-}480h}$ showed less correlation

with the ideal body weight than body weight. It was suggests that the body fat of abdomen might have influenced the absorption of etanercept because etanercept was administered by SC at abdomen in all five studies. Moreover, recently, research into the role of mesenteric fat in chronic inflammatory diseases has intersected with investigation into the importance of adipose tissue as a metabolically active source of proinflammatory cytokines [18]. RA patients with high BMI exhibited a diminished clinical response to infliximab treatment, despite drug dosing based on body weight [37]. However, the influence of the body fat on absorption is unidentifiable without intravenous (IV) dosing data. Addition studies comparing PK after IV and SC in same subjects or evaluating the relationship between PK parameters and WT after IV dosing are required.

In summary, the PK of etanercept seems to be strongly influenced by several factors not only the intrinsic factor of the subject but also the extrinsic factor such as the manufacturing process. Because this was not a controlled study for hypothesis testing, it is hard to evaluate the extent of the influence of each factor. However, when these factors influence on the PK profile in combination, maximum twofold difference of drug exposure between subjects was observed. By this difference of PK, unexpected adverse events or subtherapeutic outcome during therapy can occur in patients.

Therefore, a better understanding of the determinants of the PK of etanercept is important to ensure more efficient dosing regimens, which may in turn improve and optimize the therapeutic management of patients. Moreover, further studies that evaluate the relationship between the PK and

pharmacodynamics of etanercept are necessary to apply the results to patients in clinical practice.

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국문 초록

건강한 한국인 자원자에서

Etanercept 단회 피하투여에 의한

약동학적 특성에 관한 연구

서론: Etanercept는 류마티스 관절염과 같은 면역질환의 치료에 사용되는 tumor necrosis factor 재조합 융합 단백질이다. 본 연구는 다섯 건의 임상시험의 약동학 결과를 비교 분석하여 etanercept의 약동학 특성에 영향을 주는 인자를 탐색하고자 하였다.

방법: 다섯 건의 임상시험 결과로부터 총 169명의 피험자의 etanercept의 혈청 농도를 수집하여 비구획모형 및 구획모형을 이용하여 약동학 분석을 시행하였다. 혈액샘플은 etanercept 25 mg 피하투여 후 3주 또는 4주 동안 수집하였다. Enzyme-linked immunosorbent assay (ELISA) 을 이용하여 etanercept의 혈청 농도를 측정하였다.

결과: 비구획모형 약동학 분석 결과로 산출된 최고 혈청 농도, 혈청 농도-시간 곡선하 면적, 반감기 등의 약동학 파라미터는 다섯 건의 임상시험 간에 유의한 차이를 나타내었다. 또한, 피험자의 체중, 약

물의 제형, 그리고 임상시험간 차이(study effect)가 약동학 파라미터에 영향을 주는 것을 집단 약동학 분석으로 확인하였다. 약동학 파라미터의 임상시험간 차이(study effect)는 약물 농도 분석 방법, etanercept 제조시설의 batch의 차이 및 제조 공정의 변화에 의해 설명 되었다.

결론: Etanercept의 약동학 특성은 피험자의 체중, 약물의 제형, 그리고 제조 공정의 변화에 영향을 받는 것을 확인하였으며, 단백질 제제의 복잡성을 고려하였을 때 이러한 결과는 etanercept의 변이(variability)를 이해하여, 궁극적으로 환자 약물요법을 향상시키는데 기여할 것으로 사료된다.

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주요어: **Etanercept, 단백질 제제, 약동학, TNF 억제제**

학번: **2012-21745**